Transcriptional and Translational Reporter Genes

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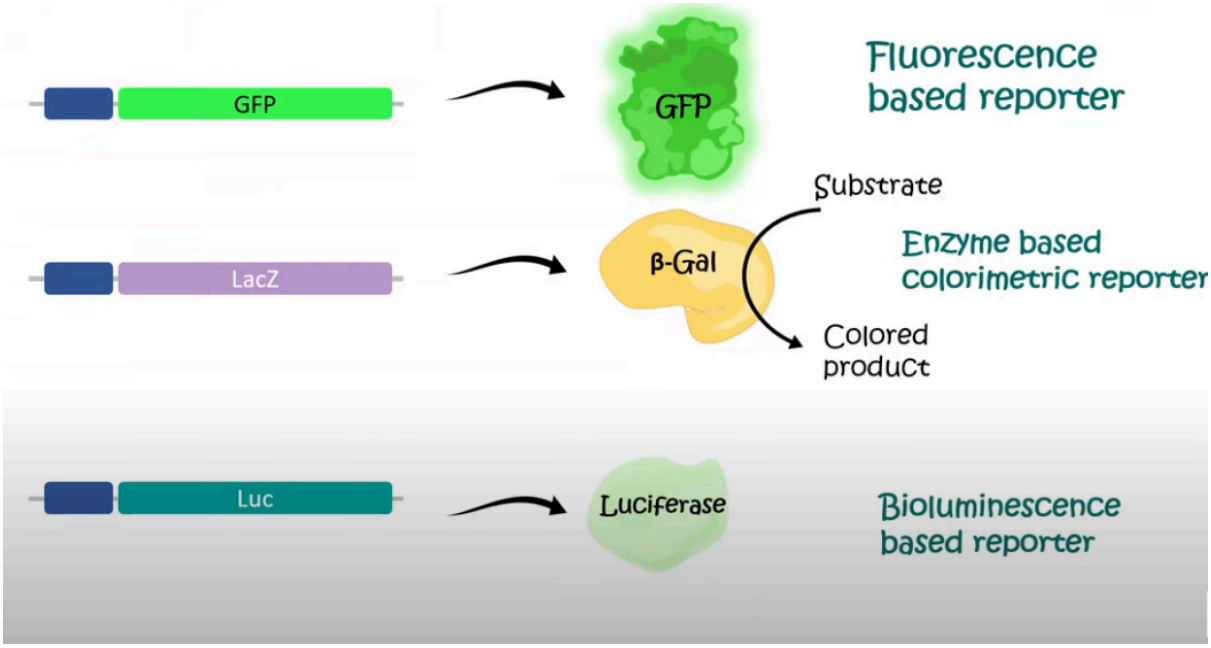
Reporter Genes

Reporter Genes are used to express a specific gene visually. The main purpose of the reporter gene assay is to investigate the promoter of a gene, whether that particular promoter is active and how that promoter is regulated. They would help us to visualize the overall rate of transcription from a promoter.

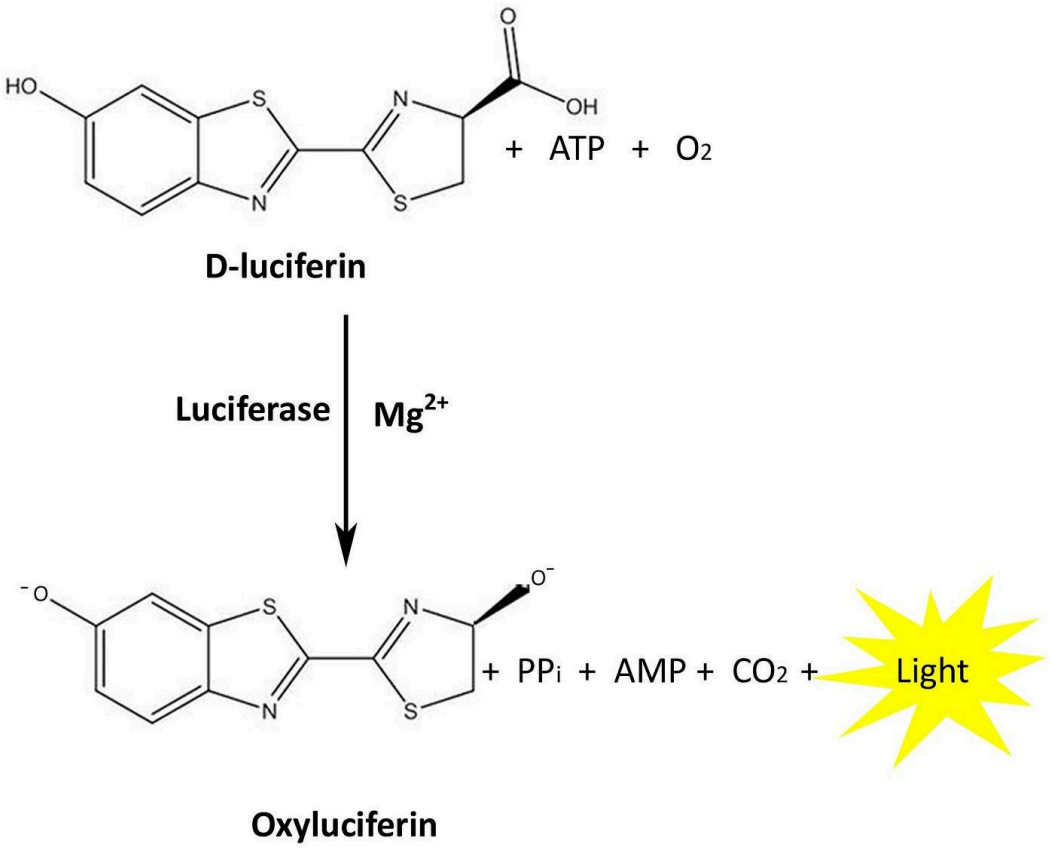


There are different types of reporter genes, and we will be diving into some of them.

1. Luciferase

Luciferase is an enzyme which gives rise to bioluminescence, and it is common in fireflies. Firefly Luciferase enzyme converts substrates like luciferin into oxyluciferin.

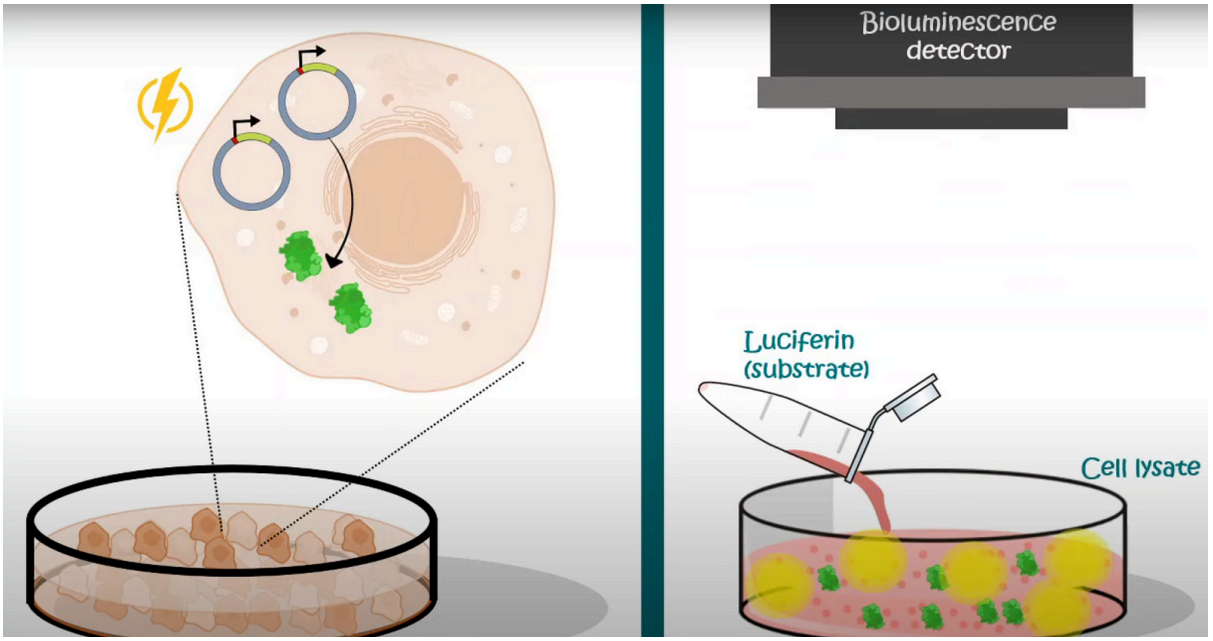
In this process, light is generated, and this is the **bioluminescence phenomenon**.



The first step is to determine if a specific promoter is **active**.

To do this, we clone the promoter upstream of a luciferase reporter sequence. If this promoter is active, it will produce mRNA for Luciferase, which will then be translated into the Luciferase enzyme. When we introduce a substrate, this enzyme will exhibit bioluminescence.

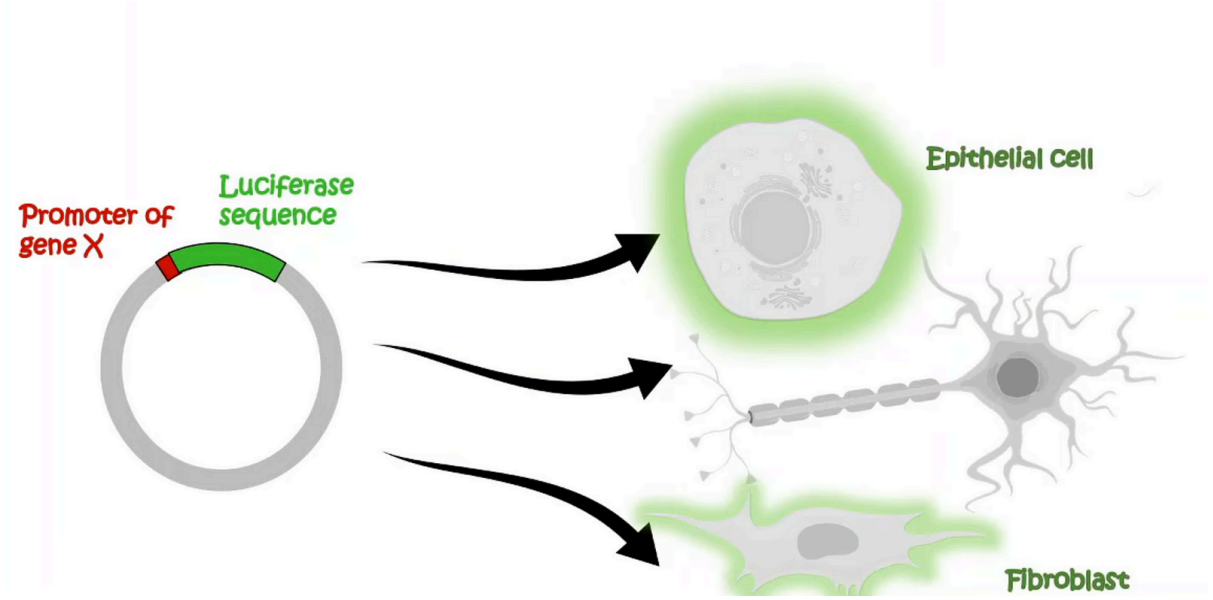
We need to transfect these expression vectors into a cell and allow some time for the luciferase enzyme to be expressed. After that, the cells are homogenized and the substrate is added. Once the substrate is introduced, the luciferase will convert luciferin into oxyluciferin, and the bioluminescence activity can be detected using a detector. This is the principle behind the luciferase reporter assay.



from "Animated biology with Arpan"

Let's go deeper into some examples. First, suppose we want to determine if a specific gene is expressed in a tissue-specific manner. We clone our promoter of interest upstream to a Luciferase reporter gene, then transfect it into various cell types and check the bioluminescence activity. This activity serves as a readout, indicating whether the gene is expressed in that particular cell type.

In the example below, bioluminescence was observed in the epithelial and fibroblast cells, but not in the neuronal cell. This suggests that this specific gene of interest is specific to epithelial or fibroblast cells and is not expressed in neurons.



The advantages of this luciferase reporter assay include its sensitivity, reproducibility, and compatibility with internal controls and reporters. These internal controls help manage variability, making the assay highly sensitive and reproducible. Additionally, this method does not use any radioactive substances, making it hazard-free.

2. Green Fluorescence Protein

Green fluorescence protein or GFP is a protein that exhibits bright green fluorescence if exposed to a blue or ultraviolet light. This green fluorescence protein has a beta-barrel-like structure (see below) and it was discovered from the jellyfish Aquaria victoria.

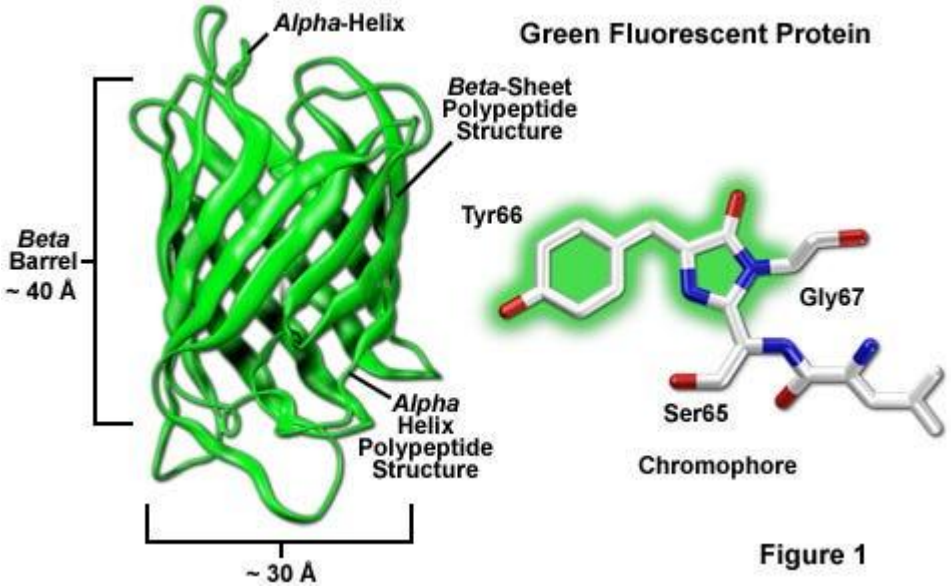
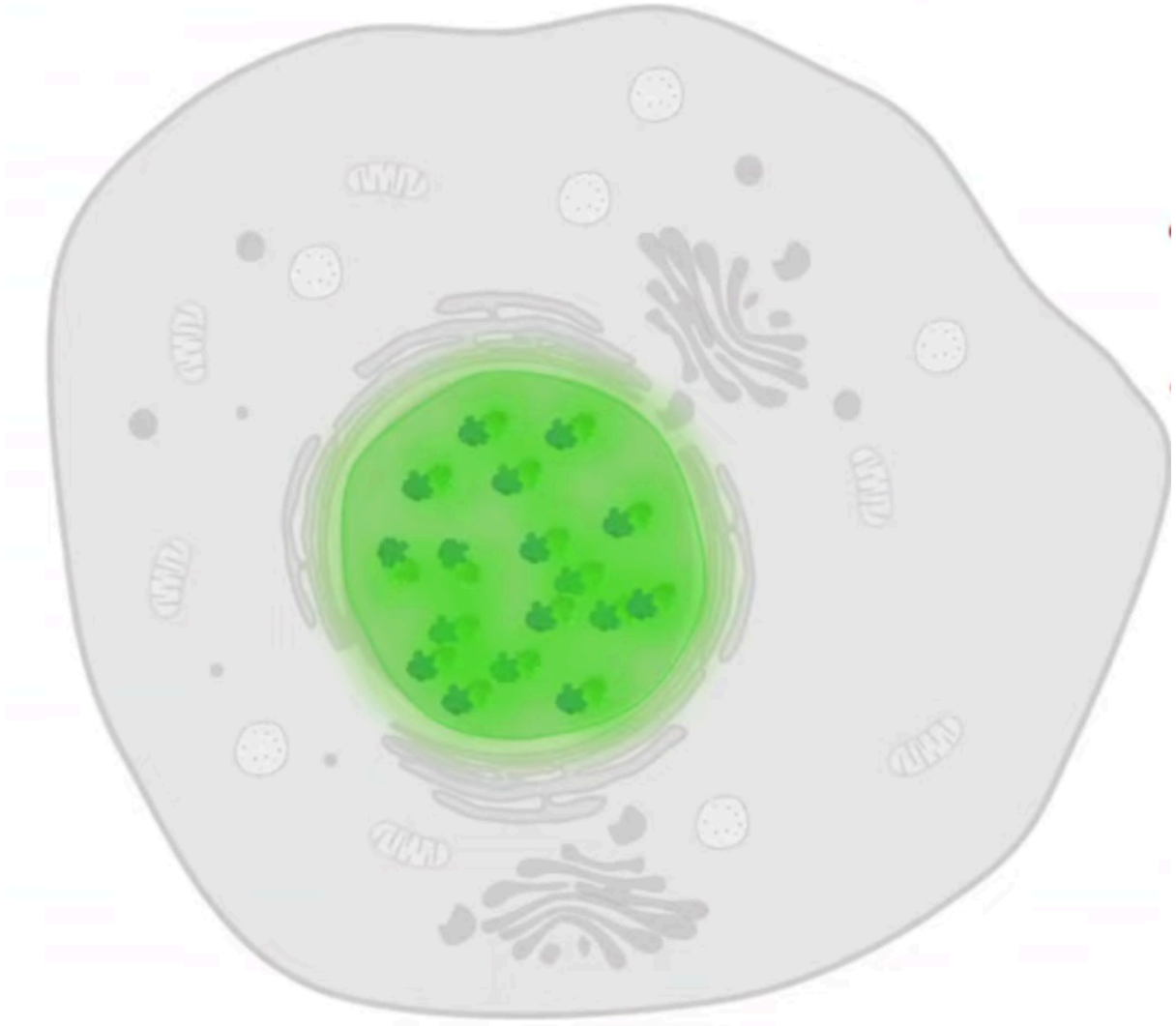


Figure 1

The main use is in determining the subcellular localization of a protein of interest, which we will refer to as protein X.

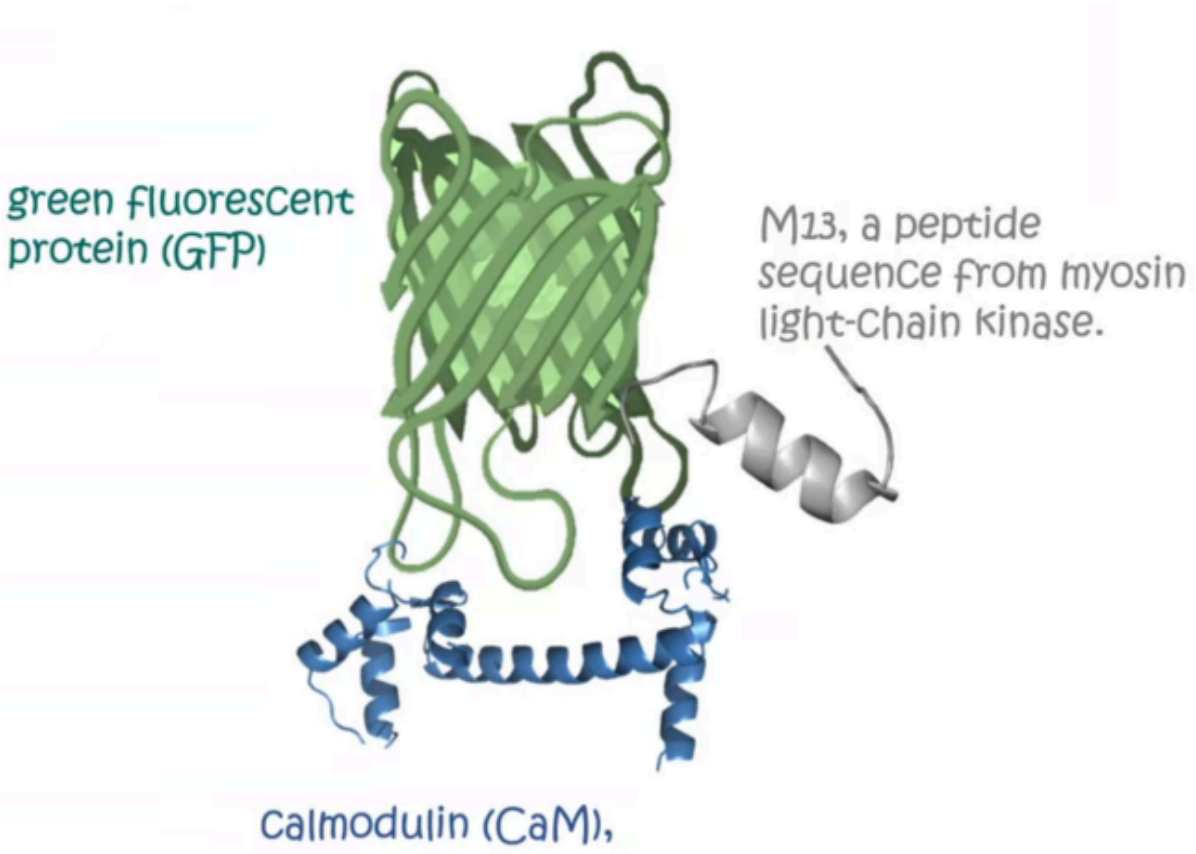
Using recombinant DNA technology, protein X is tagged with GFP. This involves cloning the coding region for protein X along with the GFP sequence into an expression vector. This vector is then transfected into a cell. When protein X is transcribed, its location can be determined by observing the fluorescence. In this case (see below), all the fluorescence comes from the nucleus, indicating that protein X is localized in the nucleus. This method is sensitive, reproducible, and does not involve any hazardous substances.



Additionally, if we want to track a specific vesicle, we can label it with Green Fluorescent Protein (GFP). If we tag a protein like RAB that is associated with the vesicle, the vesicle will also be labeled. This allows us to track the vesicle's position in real-time by following the fluorescence.

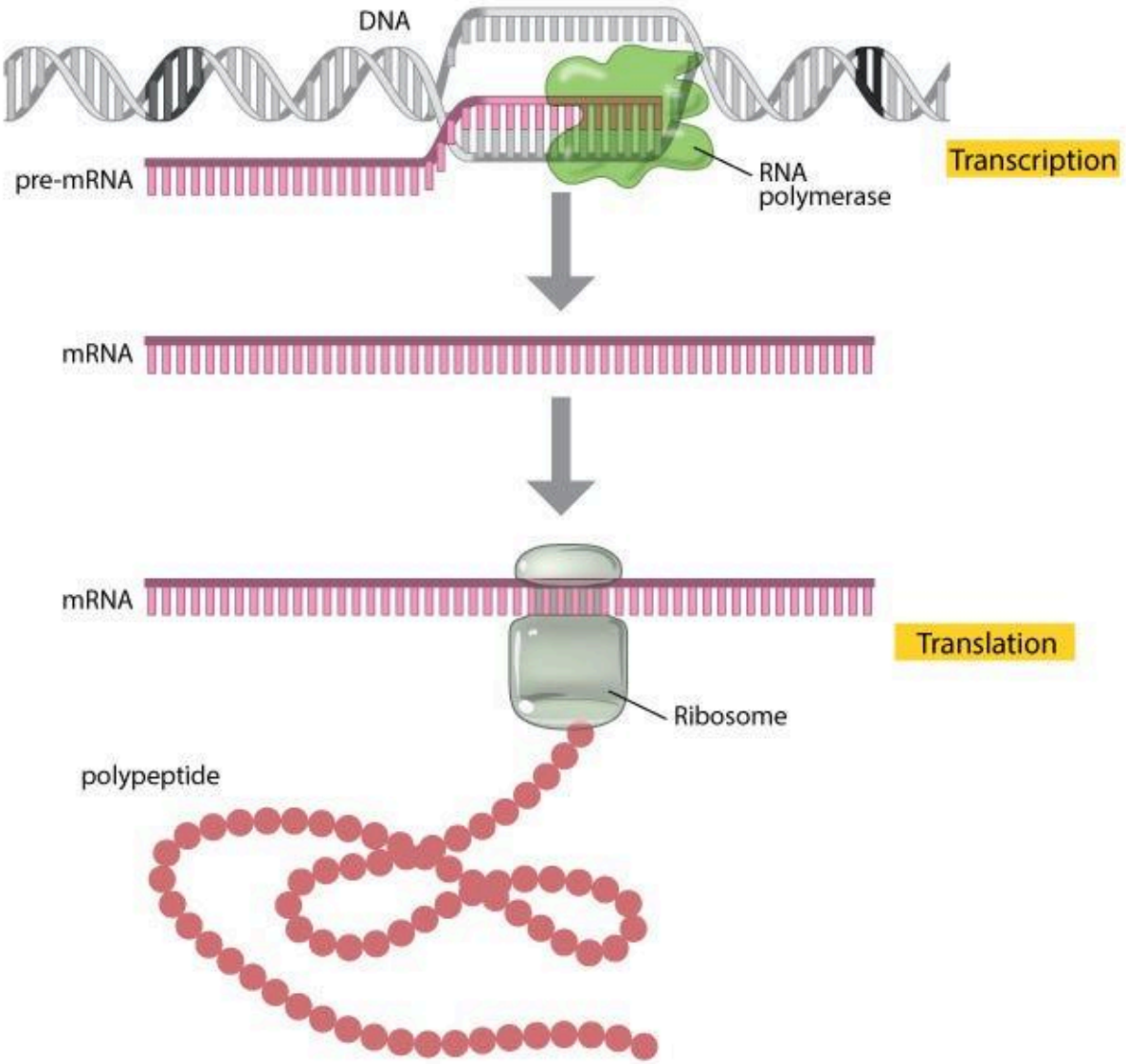
This is also especially relevant for studying the trafficking of neurotransmitter-containing vesicles in neurons. These neurotransmitters are generated in the cell body and transported to the synapse, a process that involves a long journey along microtubules. Kinesins act like vehicles on this highway, moving the vesicles to the synapses.

By labeling these vesicles with GFP, we can monitor their motion in real-time. This allows us to understand various aspects of their movement, including time scales, kinetics, and direction.



In the example above, a GFP fluorescent protein is tagged with a calmodulin protein that can bind to calcium and an M13 helix from a myosin light chain kinase. When calcium binds, a conformational change occurs, making the protein fluorescent. By observing these fluorescence levels in real time, we can understand how calcium fluctuations occur. For instance, when a neuron fires, the fluorescent intensity increases and then decreases when the neuron stops firing. This serves as an indicator of neural activity.

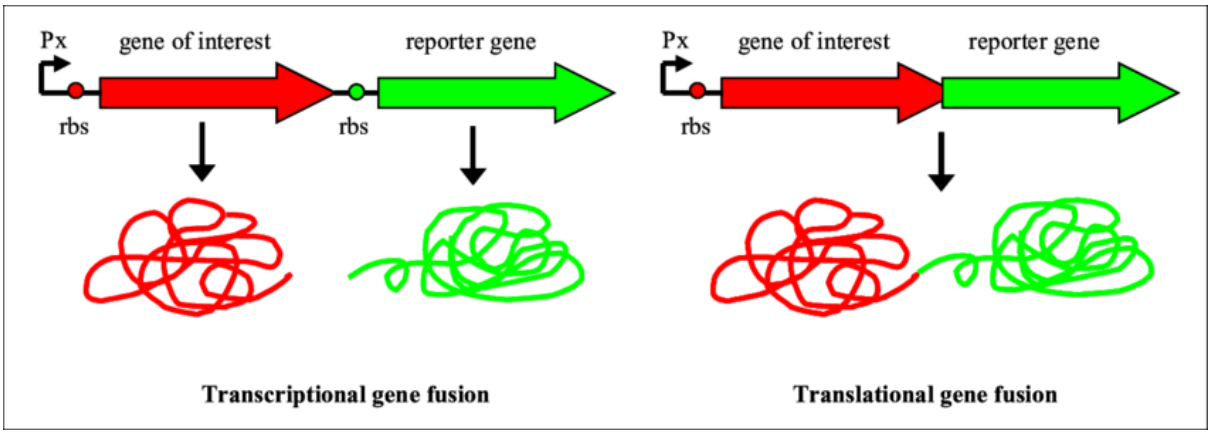
Transcriptional and Translational Genes



Reporters are genes that are fused to genes of interest, abbreviated GOI, to monitor their activity at the transcriptional or translational level. Transcriptional reporter genes are fused to the promoter of the gene of interest, which is depicted in Figure A below:

Translational reporter genes, on the other hand, are fused to the gene itself, as seen in Figure B. The reporter could be fused to just a segment of the gene or the entire gene.

As such, transcriptional reporters are fused to the promoters of genes, which provides information on where the gene is transcribed, but not where the translated protein ends up.



Translational reporters that are fused to the entire gene of interest provide information on where the translated proteins are located, and not necessarily where the RNA was transcribed.

On the other hand, transcriptional reporters are used to study gene expression by linking a gene of interest to a reporter gene. This allows researchers to observe where and when the gene is transcribed within the cell.